

Appendix J

**Standard Operating Procedures
For Algal Samples**

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PROTOCOL FOR ALGAL/PWS/NUTRIENT SAMPLE COLLECTION

(HAB-001)

I. INTRODUCTION

A. Purpose

The following paragraphs describe the procedures for the collection, preservation, and transport of algal samples from water bodies with a validated HAB complaint.

B. Minimum Staff Qualifications

Personnel implementing this SOP should meet the minimum classification requirements for environmental scientist I published by the Kansas Department of Administration. They also should be experienced in the measurement of the physicochemical and microbiological properties of surface water and in the performance of environmental field investigations.

C. Equipment/Accessories

1. Cubitainers – 1 per sample location
2. Amber Glass (TOC), Non-Preserved Bottle – 1 per BA sample location
3. Nutrient Bottle - 1 per HAB event
4. Water proof permanent marker
5. Pole sampler, bucket
6. GPS unit
7. Paper Towels
8. Cooler
9. Cold packs or ice
10. Zip lock plastic bags for ice if needed
11. Tap or distilled water
12. Trash bags
13. Map of area

PROCEDURES

A. Collection of Algal Samples - (one cubitainer per site)

1. Using a pole sampler with an attached beaker, either a “grab” or “composite” sample can be collected.
 - a. *“Grab” Samples:*
 - 1) Verify that the designated sampling location(s) where a sample is to be collected is representative of a major public access area where the public will have full access to the water.
 - 2) Mark the cubitainer (clear, plastic, 1-liter cube shaped bottle, non-preserved) with the following information:
 - a. Sample location I.D. number
 - b. Sample Collection Time
 - c. Sample Collection Date
 - d. Sample Collector’s Name or Initials
 - 3) Inflate the cubitainer. Inflating the cubitainer can be done by removing the cap, gently exhaling into the opening until it completely inflates. (Do not contaminate the cube by expelling saliva into the container.)
 - 4) Submerge the pole sampler’s beaker open end down through the water until completely covered. Slowly turn the sample pole until the beaker is sideways and approximately 1 -2” under the water’s surface. Once the beaker is filled pull the sample pole and attached beaker out of the water.
 - 5) Pour collected sample into the inflated cubitainer. It generally takes two of these processes to fill one cubitainer.
 - 6) Leave a headspace in the container of no less than ½” for gas expansion.
 - 7) Secure the lid and wipe the cubitainer until it is clean and dry. Place in a cooler.
 - 8) Move to your next sampling location.
 - 9) If sample collection is required in the future at that same public access area, then collect as close as possible to that exact initial point. (Don’t go chasing scum that might appear a few feet away from your original sampling point.) This will maintain consistency of the sampling activities.

b. “Composite” Samples:

- 1) Verify that the designated sampling location(s) where a sample is to be collected is representative of a major public access area where the public will have full access to the water.
- 2) Mark the cubitainer (clear, plastic, 1-liter cube shaped bottle, non-preserved) with the following information:
 - a. Sample location I.D. number
 - b. Sample Collection Time
 - c. Sample Collection Date
 - d. Sample Collector’s name or initials
- 3) Inflate the cubitainer. Inflating the cubitainer can be done by removing the cap, gently exhaling into the opening until it completely inflates. (Do not contaminate the cube by expelling saliva into the container.)
- 4) Submerge the pole sampler’s beaker open end down through the water until completely covered. Slowly turn the sample pole until the beaker is sideways and approximately 1 -2” under the water’s surface. Once the beaker is filled pull the sample pole and attached beaker out of the water.
- 5) Pour collected sample into a clean bucket. Continue this process collecting samples from various locations around the dock, (e.g., one from each side of the dock).
- 6) Once all samples have been collected and poured into the bucket, swirl the bucket to mix.
- 7) Pour the mixed sample into the inflated cubitainer.
- 8) Leave a headspace in the container of no less than ½” for gas expansion.
- 9) If sample collection is required in the future at that same public access area, then collect as close as possible to that exact initial point. (Don’t go chasing scum that might appear a few feet away from your original sampling point.) This will maintain consistency of the sampling activities.

B. How to Collect Water Samples for Drinking Water Analysis – (one glass TOC bottle):

1. Using a pole sampler with an attached beaker or bucket with rope and swivel, a BA sample can be collected.

2. Verify that the designated sampling location(s) is representative of where a public water supply system would be pulling their water.
 3. Mark the sample bottle (amber, glass, TOC bottle, non-preserved) with the following information:
 - a. Sample location I.D. number
 - b. Sample Collection Time
 - c. Sample Collection Date
 - d. Sample Collector's Name or Initials
 4. Remove the cap.
 5. Submerge the pole sampler's beaker open end down through the water until completely covered. Slowly turn the sample pole until the beaker is sideways and approximately 1 -2" under the water's surface. Once the beaker is filled pull the sample pole and attached beaker out of the water. Do the same process for a bucket and rope.
 6. Pour collected sample into the glass bottle.
 7. Leave a headspace in the container of no less than ½" for gas expansion.
 8. Secure the lid and wipe the bottle until it is clean and dry. Place in a cooler.
 9. Move to your next sampling location.
 10. If sample collection is required in the future at that same public access area, then collect as close as possible to that exact initial point. (Don't go chasing scum that might appear a few feet away from your original sampling point.) This will maintain consistency of the sampling activities.
- C. How To Collect Nutrient Samples - (one Cubitainer and one Nutrient bottle per site):**
1. Using a pole sampler with an attached beaker, a "grab" sample can be collected.
 - a. Nutrient samples will be collected only from designated locations believed representative of the lakes normal ambient sampling station. Verify that the location where the sample is to be collected is at the normal ambient sampling station or as close as possible to that station location.

2. Mark the nutrient container, (brown, opaque, plastic 250 milliliter bottle, Sulfuric Acid preserved) and a cubitainer, with the following information:
 - a. Sample location I.D. number
 - b. Sample Collection Time
 - c. Sample Collection Date
 - d. Sample Collector's name or initials
3. Submerge the beaker's open end down through the water until completely covered. Slowly turn the sample pole until the beaker is sideways and approximately 1 -2" under the water's surface. Once the beaker is filled pull the sample pole and attached beaker out of the water.
4. Carefully open the Nutrient bottle. (The bottle contains a Sulfuric Acid preservative and will burn the skin or clothing.)
5. Slowly pour collected sample into the bottle making sure not to overfill as a loss of preservative will affect the integrity of the sample.
6. Secure the lid tightly and wipe clean and dry. Place the sample container in a zip lock bag and secure bag. (Placing the container in its own individual bag will minimize the potential of cross contamination).
7. Follow the "[How to Collect Phytoplankton Samples](#)" directions to fill the cubitainer for nutrients.
8. Place containers in a cooler.

*****REMINDER: All samples collected should be taken 1 to 2 inches under the water surface. Samples do not need to be taken at deeper depths when sampling for blue-green algae. Do not collect the sample at the surface (the air/water interface).**

D. Cleaning Equipment Between Samples:

1. "Between Sampling Locations at the Same Water Body"
 - a. After the sample has been collected, discard any remaining water from sampling equipment.
 - b. Wipe all equipment dry with paper towel
 - c. Once at the next sampling location, rinse sampling equipment with ambient water at a different, but nearby, location than where the sample is to be collected.

- d. Discard rinsate water then move to sampling location to collect sample.

2. "Between Different Water Bodies"

- a. Clean all sampling equipment with tap water.
- b. Once at sampling location, rinse sampling equipment several times with ambient water at a different, but nearby, location than where the sample is to be collected.
- c. Discard rinsate water then move to sampling location to collect sample.
- d. Follow the above directions if additional samples are to be collected at the same waterbody.

E. Preparing Samples for Shipment:

- 1. Wipe dry the exterior of all cubitainers.
- 2. Place all containers in a cooler along with a cold pack(s). If ice is used, double bag it first before placing in the cooler. This will minimize the potential of cross-contamination or making a mess when the ice melts. Plastic pop bottles filled with water and then frozen can also be used in place of cold packs. Place enough cold packs or ice in the cooler to keep the samples cool. **DO NOT FREEZE THE SAMPLES.**
- 3. Fill out the algae and/or the KHEL PWS submission/chain-of-custody form (see Appendix E, p.61). When filling out the algal form, include environmental conditions at time of sampling, e.g. water and air temperature, wind speed/direction, cloud cover, etc.
- 4. If the samples are to be sent by a carrier company, then place the submission form(s) inside a plastic bag and tape inside the cooler on its lid.
- 5. If samples are to be transported to the lab(s) by the collector or handed off to another partner, then keep the forms out and readily available to be signed by the next handler.
- 6. Secure the cooler.

D. Shipment of Samples:

- 1. Samples must be shipped no later than overnight.
- 2. At any time that the samples exchange to a different handler, then the chain-of-custody must be signed. Exception is if shipment is sent by commercial carrier, then note what company the samples were "relinquished to ..."

3. All samples are to be sent to:

BOW/BEFS
1000 SW Jackson
Suite 440
Topeka, Kansas 66612

4. All nutrient samples are to be sent to the:

KHEL Lab
Forbes Field, Building 740
Topeka, Ks. 66620-0001

5. All Public Drinking Water samples will be taken to the Kansas Health and Environmental Lab by BOW/PWS staff.

If samples are shipped by carrier, to minimize shipping costs, send all samples to BOW/BEFS, including nutrient samples and we will carry the nutrient samples to the lab.

Further question? Contact:

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Layne Knight – (785) 291-3885
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INITIAL PROCESSING OF ALGAL SAMPLES (HAB – 002)

I. INTRODUCTION

A. Purpose

The following paragraphs describe the procedure for the collection, preservation, and transport of algal samples from water bodies with a validated HAB complaint.

B. Minimum Staff Qualifications

Personnel implementing this SOP should be experienced in the measurement of the physicochemical and microbiological properties of surface water and in the performance of environmental field investigations.

C. Equipment/Accessories

1. Lugol's solution
2. Sample bottles with lids
3. Yellow round labels
4. Chain of Custody sheet.

II. PROCEDURES

A. Receiving samples

1. Coolers containing samples will be directed to BOW/BEFS. This is usually around 10:00 to 10:30 on Tuesday or Wednesday depending upon the carrier bringing in the samples.
2. Upon receiving the coolers, remove the samples and paperwork.
3. Place samples in refrigerator in the Algal ID lab until ready to process. Make sure the refrigerator door closes completely.
4. On the back of the Algae Sample Submission Form (see Appendix E, p.61) and under the Chain of Custody section, date and sign by the Received By line.
5. The Algae Sample Submission Forms are to be kept with the samples and are placed by the algal taxonomist's scope. Once sample analysis is completed, then the Sample Submission Form is to be filed by year with the lake reports.

B. Algal Preservation

1. When ready to process samples, remove them from the refrigerator and place on the black shelving unit in middle of Algal ID lab room in the order they will be processed in.
2. In the drawer directly to the left of the sink, remove the yellow, round stickers and a pencil to mark each bottle used to preserve samples.
3. In the chlorophyll lab room (small room) and directly to the left of the door on a cart, remove one bottle for each sample to be preserved. Lids to the bottles are in the far-right bottom cabinet and on the bottom self.
4. Line the bottles and lids up on the counter by the refrigerator in the Algal ID lab. Write on the yellow stickers the lake number and sampling point (for example – Milford would be 190 as the lake number and AA is one of the sample points). Place the stickers on top of the lids prior to preserving sample.
5. In the same room and under the sink is the brown Lugol's container. It has a small needleless syringe attached to it. Place 5 drops of Lugol's in each bottle. Be careful with this as Lugol's does stain just about everything it comes in contact with, including the counter, hands, and clothes. Place bottle back under the sink.
6. Take the first sample and shake cubitainer vigorously to thoroughly mix the water and contents. Over the sink, carefully pour sample water into one of the small bottles containing the Lugol's until it is about to the line where the neck begins (about $\frac{1}{4}$ inch from the top). Double check that the lid you put on the bottle matches the sample you poured into it. Screw that lid onto the bottle securely.
7. A portion of the sample will need to be frozen to conduct the Elisa toxicity test so pour a good portion of the sample down the drain but retain at least a $\frac{1}{2}$ inch in the bottom of the cubitainer.
8. Continue this process with all the remaining samples.
9. Place the preserved samples on the table with the older scope. This scope has a black trash bag over the scope.
10. Place the cubitainers with the saved water into the freezer in the main lab (middle) room. Make sure the lid to the freezer is closed tightly.

MICROCYSTIN TEST USING *ELISA QUALITUBE KIT (ET 022) (HAB – 003)

I. INTRODUCTION

A. Purpose

The following paragraphs describe the procedures for the collection, preservation, and transport of algal samples from water bodies with a validated HAB complaint.

B. Minimum Staff Qualifications

Personnel implementing this SOP should be experienced in the measurement of the physicochemical and microbiological properties of surface water and in the performance of environmental field investigations.

C. Equipment/Accessories

1. Envirologix qualitube kit for Microcystin.
2. Wooden block to place tubes in for analysis.
3. Timer.
4. Use of water in sink area.
5. Thawed water samples from freezer of lakes that are under investigation.
6. Paper and pencil.

II. PROCEDURES

A. Preliminary procedures:

1. Remove frozen samples from freezer to begin thawing.
2. Read all the instructions before running the kit.
3. Allow all reagents to reach room temperature before beginning (at least 30 minutes with un-boxed tubes and reagents at room temperature – do not remove tubes from bag with desiccant until they have warmed up).
4. Organize all samples and reagents so that steps 1 and 2 can be performed in 3 minutes or less.
5. Do not run more than 6 tubes at a time.

B. Completing the assay.

1. Place tubes in wooden tube rack. The Calibrator tubes are the two tubes behind the six in front. These will be the tubes used to compare color intensity between 0.5 ppb and 3.0 ppb results. See Photo below.



2. Rapidly add **5 drops** of Microcystin Assay **diluent** to each tube in the assay.
3. Using the sample pipette provided, immediately add **2 drops of 0.5 ppb Microcystin calibrator** to the first tube. Add 2 drops of 3.0 ppb **Microcystin calibrator** into the second tube. Add 2 drops of sample to each of the subsequent tubes up to a total of 4 samples. **Do Not Add Microcystin-enzyme conjugate in this step.**
4. Thoroughly mix the contents of the tubes by moving the tube holder in a rapid circular motion on flat surface for a full 20 - 30 seconds.
5. Incubate tubes at ambient temperature for 5 minutes.
6. Add **5 drops of Microcystin-enzyme conjugate** to each tube. Do not empty the tub contents or wash the tubes at this time. Thoroughly mix the contents of the tubes as in step 3.
7. Incubate tubes at ambient temperature for 20 minutes.
8. After incubation, vigorously shake the contents of the tubes into a sink or other suitable container. Flood the tubes completely with cool tap water, and then shake to empty. Repeat this wash step 3 times. Invert the tubes on a paper towel and tap to remove as much water at possible.

9. Add **10 drops of Substrate** to each tube. Thoroughly mix the contents of the tubes, as in Step 3. Incubate substrate in tubes for 10 minutes at ambient temperature.

NOTE: If blue color does not develop in the 0.5 ppb Calibrator tube, the assay is invalid and should be repeated.

10. This assay is designed to be read visually with un-stopped tubes (blue solution). If tubes are to be read using the tube photometer pipette 0.7 mL of Stop Solution into each tube and mix thoroughly. This will turn the tube contents yellow.

- a. Tubes should be read **within 30 minutes** of the addition of Stop Solution.

11. Interpret the results of un-stopped tubes immediately following the 10-minute substrate incubation.

C. Interpreting the results

1. Compare the intensity of the blue color of each sample tube to the intensity of the blue color in the 0.5 and 3.0 ppb calibrator tubes.
2. Score each sample tube as having less than, more than or equal color to the two calibrator tubes.
3. Use the Table below to determine the level of microcystin in the samples.

Samples with Optical Density (OD) values.....	Contain.....
Greater than OD of 0.5 ppb Calibrator	Less than 0.5 ppb Microcystin
Between OD of 0.5 ppb and 3.0 ppb Calibrator	Between 0.5 and 3.0 ppb Microcystin
Less than OD of 3.0 ppb Calibrator	More than 3 ppb Microcystin

EnviroLogix® ELISA QualiTube assays were used according to manufacturer's specifications to measure total microcystin (detection limit 0.5 µg/L), viewable online at: <http://www.envirologix.com/wp-content/uploads/2015/05/ET022-Microcystin-101215.pdf>. EnviroLogix, Portland, Maine.

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